The Canadian
Medical Association Journal

Vol. XXVII TORONTO, NOVEMBER, 1932 No. 5

THE INFLUENCE OF GASTRIC MUCUS ON PEPTIC DIGESTION

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THE gastric mucus plays a manifold part. It protects the mucous membrane from injury (Zweig[28]), it neutralizes the hydrochloric acid of the gastric juice at the beginning and at the end of secretion when the flow of juice is scanty (Heidenhain,[13] Pavlov and Schumow-Simanowski,[19] Webster,[24] Bolton and Goodhart,[8]) and it delays the diffusion of hydrochloric acid and pepsin, thus protecting the mucosa from autodigestion (Whitlow, see Fogelson[12]). In the case of irritation of the gastric mucosa by chemical agents (concentrated solutions of alcohol, silver nitrate, sublimate, etc. an abundant secretion of thin mucus ensues which by diluting the irritant solution diminishes its damaging effect (see Babkin[2]).

Many investigators, both physiologists and clinicians, have contributed to our knowledge concerning the rôle of the gastric mucus in gastric digestion. This problem has also been studied in our laboratory during the last few years (Babkin,[3,4,5] Webster,[24,25,26] Webster and Komarov,[27] Babkin and Komarov[6]). At present we have to discriminate between two kinds of mucus secreted by the gastric mucosa—surface epithelium mucus and "dissolved mucin." The latter is present in a dissolved state in pure acid gastric juice. Its chemical properties are quite different from those of surface epithelium mucus, and it is probably secreted not by the surface epithelium but by glandular epithelium (Webster and Komarov[27]).

Since the gastric mucus has the power of combining with free acid, and since it may serve as a protective coating for ulcers, Kim and Ivy[15] and Fogelson[12] conceived the idea of using it as a protection against further destruction of the gastric and intestinal walls in cases of peptic ulcer. It is yet early to judge the results of such a recently introduced method of ulcer treatment in patients, but already there are reports of its successful application (Atkinson,[1] Fogelson—at the Meeting of the American Gastro-Enterological Association, May 2, 1932) as well as some adverse criticism (Rivers, Vanzant and Essex,[21] Leon Bloch—at the Meeting of the American Gastro-Enterological Association, May 2, 1932). Further clinical investigations on a wider scale will reveal its true therapeutic value.

We approached the problem of the protective action of mucin from another angle. If gastric juice is able to produce lesions of the mucous membrane, or digest further an already existing ulcer, it does so not only because it is acid but because it possesses a proteolytic enzyme acting in an acid medium. Therefore the question naturally arises—how does mucus affect peptic digestion? There are no direct indications in the literature with regard either to the influence of mucus on peptic digestion or to the action of organic substances in general, even such as the products of protein disintegration (Rona and Weber[22]). However, in the literature on anti-pepsin some indications are to be found concerning the part played by the mucus in preventing autodigestion of the gastric mucosa and destruction of the intestinal mucous membrance by pepsin (Roux,[23] Klug,[16] Dezani,[11] Best[7]).

It was therefore decided to study the effect of different mucus preparations, mucoproteins and their derivatives on peptic digestion. The following substances were investigated: gastric surface epithelium mucus; dissolved gastric mucoprotein; different preparations of submaxillary mucus ob-

tained from freshly secreted dog's saliva. A considerable part of the work was performed with mucus preparations from the hog's stomach, which were kindly sent to us by Dr. S. J. Fogelson, to whom we here express our thanks. This preparation, which Dr. Fogelson calls "Gastric Mucin," is obtainable in the form of a dry powder. One gram of the preparation combines with about 15 c.c. of 0.5 per cent hydrochloric acid (Fogelson[12]). Owing to the method employed in the preparation of "Gastric Mucin," *viz.*, incubation of hog's gastric mucous membrane with dilute hydrochloric acid and precipitation of the extract obtained with an equal volume of alcohol (Fogelson,[12]) it cannot be regarded as a pure substance. It was found to contain peptones and other products of incomplete protein digestion (Jones and Ivy,[14]) and also probably histamine (Rivers, Vanzant and Essex[21]).

Two samples of Fogelson's "Gastric Mucin" which we studied were not quite pure. They were not clearly soluble in water, in a weak alkaline solution, or in 0.2 per cent HCl. About 5 per cent of the total weight of the substance could be extracted by absolute alcohol. Some substances also passed into the absolute acetone and absolute ether extracts made. On treatment of a watery suspension of "Gastric Mucin" with barium salts ($BaCl_2$, $Ba(OH)_2$, $BaCO_3$) a water-insoluble precipitate was formed. When this precipitate was treated with a weak HCl solution, fatty substances could be extracted with ether in the presence of some alcohol. After evaporation of the ether and alcohol, crystals of fatty acid were formed which were visible under the microscope. The biuret test was positive. A considerable amount of inorganic material, insoluble even in normal HCl, was present in "Gastric Mucin." Both samples had a secretagogue effect on the stomach. In our experiments, therefore, we used Fogelson's preparation both in the original state and after attempting to purify it in several ways. As a result of these attempts several "fractions" were obtained, none of which however consisted of pure mucin, all being contaminated with some unknown substances. A description of the preparation of these fractions will be given under "Methods."

METHODS

In all the experiments pure gastric juice was used, obtained from dogs with different pouches or œsophagotomy and a gastric fistula. Different substances were added to the juice in order to study their effect on peptic digestion. The peptic power of the juice was estimated by Mett's method with Christiansen's modification. The Mett tubes were incubated at 38°C., readings being usually taken after 24 hours, sometimes followed by further readings after 36 and 48 hours. The millimetres of the readings are given, squared, in the tables. Mett's method was selected, it being still one of the most convenient and accurate methods of determining the peptic power. This belief is supported by the recent investigation of Patterson and Adler.[18*]

All the substances studied by us lowered the acidity, particularly the free acidity, of the gastric juice, and so would influence the peptic digestion. Care was taken to adjust the acidity of the different samples and to check the neutralizing effect of the investigated substances on peptic digestion by comparison with control samples of gastric juice to which n/50 NaOH or a phosphate mixture was added. The latter was composed of 18.75 c.c. of n/5 KH_2PO_4 and 23.25 c.c. of n/5 NaOH, diluted with distilled water to 100 c.c., and was used in all the experiments quoted in the tables below. As a rule the free acidity (Töpfer's reagent) and total acidity (phenolphthalein) were determined before and after the incubation of the samples.

As was stated above, a considerable number of experiments was performed with fractions obtained from Fogelson's preparation, with alcohol as the chief reagent. Whenever necessary during the procedure, the pH of the mixture was adjusted with 12 per cent HCl or 10 per cent NaOH. Four fractions were obtained in the following way from 50 g. of Fogelson's "Gastric Mucin:"

Fraction 1 remaining after fourfold extraction of "Gastric Mucin" with 500 c.c. of 60 per cent ethyl alcohol each time. This was then extracted with 80 per cent, 95 per cent and absolute alcohol and ether. Yield 26.5 g. (53 per cent). A grey, light powder, not completely soluble in water, alkali or 0.2 per cent HCl; solutions viscous and sticky. Analysis:—biuret test negative; Millon's, xanthoprotein, and Molisch tests positive; ash, 5.6 per cent; nitrogen calculated for ash-free substance (micro-Kjeldahl method), 8.5 per cent; initial reduction (without hydrolysis) 4.4 per cent glucose; additional reduction (after 2½ hours' hydrolysis with 2 NH_2SO_4) calculated for ash-free substance, 44.0 per cent glucose.

Fraction 2 precipitate formed on adding 12 per cent HCl to the combined alcohol extracts mentioned above, until a slightly positive test to Congo red was obtained. This was filtered, washed with alcohol, acetone and ether, and dried *in vacuo*. Yield, 10 g. (20 per cent). A white, light powder, easily and completely soluble in weak alkali and 0.2 per cent HCl; not so readily but still completely soluble in water. In all cases a more or less viscous and sticky solution was obtained. Analysis:—biuret test negative; Millon's, xanthoprotein and Molisch tests positive; ash, 1.13 per cent; nitrogen, 9.3 per cent; initial reduction, 4.2 per cent; reduction after hydrolysis, 34.5 per cent glucose.

Fraction 3 was obtained from the combined alcohol filtrates left after the first and second fractions. These were evaporated *in vacuo* after neutralization with NaOH. The syrupy residue was extracted with n/50 NaOH at pH 8.0, and the clear extract so obtained was precipitated with 9 volumes of absolute alcohol. This precipitate was filtered, washed with alcohol, acetone and ether, and dried *in vacuo*. Yield about 2 g. (4 per cent). A yellowish-grey powder, very easily and clearly soluble in water. The solution was not viscous. Analysis:—biuret test distinctly positive; Millon's and xanthoprotein tests slightly positive, less than in fractions 1 and 2; Molisch test positive; ash, 15.5 per cent; nitrogen calculated for ash-free substance, 9.64 per cent; initial reduction 4.0 per cent; additional reduction after hydrolysis, 31.5 per cent.

Fraction 4 represents all the substances clearly soluble in water which could be extracted from Fogelson's

* After this paper went to press, similar results were obtained by us when digestive power was estimated by the increase of non-protein nitrogen, casein (Merck, according to Hammarsten) being used as a substrate.

preparation with 90 per cent alcohol made slightly acid to litmus but negative to Congo red. The alcohol was evaporated and the fluid was concentrated by neutral reaction. When Fraction 4, in an amount corresponding to 20 g. of Fogelson's original preparation, dissolved in 200 c.c. of water, was introduced into the stomach of a dog, it activated a secretion in a gastric pouch. In 30 minutes 4.2 c.c. of gastric juice were secreted, having a free acidity of 140 c.c., a total acidity of 124 c.c. n/10 NaOH, and a digestive power of 27 Mett's units. Introduction of an equal amount of water gave in one hour 1.5 c.c. of gastric juice, with free acidity 110 c.c., total acidity 90 c.c. n/10 NaOH, and digestive power 46 Mett's units.

An absolute alcohol extract was also prepared separately from Fogelson's "Mucin." It consisted chiefly of fatty acids and soaps. The crystals of fatty acid were visible under the microscope. The remainder after extraction with absolute alcohol (yield 95 per cent) was tested for its effect on peptic digestion.

The low nitrogen content and very high reduction after hydrolysis suggest that in none of our Fractions (1, 2 and 3) was a pure mucoprotein obtained. Since the "surface epithelium gastric mucin" (Webster and Komarov[27]) contained 12.45 per cent nitrogen, and its reduction after hydrolysis was equal to 30 to 35 per cent of glucose, it is reasonable to believe that the original Fogelson preparation, as well as our Fractions 1, 2 and 3, consist of surface epithelium mucus contaminated to a large extent with mucoitin-sulphuric acid and other substances.

Since in Fogelson's "Gastric Mucin," and also in our fractions, ash was present, containing sodium, potassium and calcium salts, control experiments with different salts were made. To establish as clearly as possible that the changes in the peptic power of the gastric juice were really due to the specific interaction of pepsin and substances contained in the mucus, and not merely to occasional factors, various forms of experiment were employed. The corresponding data will be given in the experimental part. Here it is only necessary to mention that the substances investigated were in some experiments added directly to the gastric juice, while in other cases they were previously dissolved in distilled water, 0.2 per cent HCl, or n/50 NaOH. The Mett tubes were usually put immediately into the samples, which were placed at once in the incubator. In some special cases the tubes were not added till several hours after the preparation of the mixtures.

EXPERIMENTAL RESULTS

In Table I results are presented of an experiment in which the effects of Fogelson's original preparation, Fractions 1 and 2 and phosphate mixture were tried on peptic digestion. The total acidity of the different samples before incubation was practically the same in all cases, whereas the free acidity varied greatly. The unpurified commercial preparation of Fogelson possessed the greatest acid-combining power. All three preparations, especially Fogelson's "Mucin," inhibited the action of pepsin. The stronger inhibitory effect of Fogelson's preparation was undoubtedly due partly to the lower free acidity of the mixture, partly to some impurities such as peptone, soap, inorganic salts, etc., which it contained. The phosphate mixture produced hardly any effect on digestion.

Table II shows that all three fractions markedly inhibited peptic digestion, the total and free acidity (shown in each vertical column) being very close in this experiment. The addition of egg-albumen (Merck) was favourable to the action of pepsin, in spite of its greater acid-combining power. On the other hand, the absolute alcohol extract from 4.2 g. of Fogelson's preparation very strongly inhibited peptic digestion, exhibiting at the same time a very pronounced buffer action. This indicates that the antipeptic and neutralizing action of Fogelson's original preparation is partly due, as mentioned above, to the presence of soap therein.

TABLE I.

EXPERIMENT, MARCH 26, 1932.

Sample	*To 10 c.c. gastric juice added:*	*Acidity in c.c. n/10 NaOH before digestion*		*Acidity in c.c. n/10 NaOH after digestion*		*Peptic power, square of mm.*
		Total	*Free*	*Total*	*Free*	
Control	10 c.c. H_2O.....	53	44	53	44	49
1	10 c.c. phosphate mixture....	54	30	54	30	45
2	330 mg. Fogelson's "Mucin" and 10 c.c. H_2O.....	54	16	58	16	12
3	330 mg. Fraction 1 and 10 c.c. H_2O.....	55	32	58	30	20
4	330 mg. Fraction 2 and 10 c.c. H_2O.....	55	32	61	30	21

N.B.—0.5 g. of Fogelson's "Mucin," of Fraction 1 and of Fraction 2 respectively were diluted with 10 c.c. of water. Five drops of phenolphthalein were added and by means of n/10 NaOH the reaction was adjusted to pH 8.0. The pH of the phosphate mixture was 8, and the reaction of water in the control sample was adjusted to pH 8 with one drop of n/10 NaOH. The volumes of the samples were made up with distilled water equal to 15 c.c. To 10 c.c. of each mixture 10 c.c. of gastric juice were added as indicated in the table.

Table III shows an experiment in which a study was made of the effect of different salts on peptic digestion. Only NaCl solution produced inhibition of peptic action, and in a mild degree. In another experiment 1 c.c. and 2 c.c. (10 and 20 mg. of the substance) of corresponding salt solutions were used. This made very little difference in the end result. Fogelson's "Mucin" when purified inhibited the peptic digestion somewhat less than in its original state (*cf.* Table I). The reason for using the sodium salt of chondroitin-sulphuric acid will be explained later on.

The following experiment on a dog with a gastric fistula and an Armour gastric pouch (formed from part of the stomach wall close to the lesser curvature) shows that the inhibitory effect of mucus on peptic digestion can be seen not only *in vitro* but also *in vivo*.

Fifteen g. of Fogelson's "Mucin" in 300 c.c. of water were introduced into the main stomach. The secretion was observed in the pouch. It lasted 1½ hours and amounted to 5.0 c.c. of gastric juice, with a total acidity of 125 c.c. and free acidity of 108 c.c. of n/10 NaOH. The digestive power in mm., squared, was 21.4. Half an hour (sample 1), three-quarters of an hour (sample 2) and one hour and a half (sample 3) after the beginning of the experiment the gastric contents were quickly removed from the stomach through the fistula, and measured; a few c.c. were subtracted for testing, and the remainder at once reintroduced into the stomach. The total acidity of the first sample was 47 c.c., the free acidity 0 of n/10 NaOH, and the digestive power in mm., squared, 1.2. The corresponding figures for the second sample were: total acidity 87 c.c.; free acidity 33 c.c.; and digestive power in mm., squared, 4.8. For the third sample the figures were: total acidity, 67 c.c.; free acidity 28 c.c.; digestive power in mm., squared, 5.8.

TABLE II.

EXPERIMENT, MARCH 15, 1932.

Sample	*To 10 c.c. gastric juice added 5 c.c. water and:*	*Acidity in c.c. n/10 NaOH before digestion*		*Acidity in c.c. n/10 NaOH after digestion*		*Peptic power, square of mm.*
		Total	*Free*	*Total*	*Free*	
Control		95	85	95	85	46
1	100 mg. Fraction 1.....	97	82	97	82	29
2	100 mg. Fraction 2.....	98	82	98	82	23
3	100 mg. Fraction 3.....	98	77	98	76	23
4	100 mg. egg-albumin.....	98	76	102	72	69
5	220 mg. absolute alcohol extract.....	140	0	140	0	29

TABLE III.

EXPERIMENT, JUNE 26, 1932.

Sample	*To 5 c.c. gastric juice added:*	*Acidity in c.c. n/10 NaOH*		*Peptic power, square of mm.*
		Total	*Free*	
Control	10 c.c. distilled water.....	50	38	22.1
1	1 c.c. 1 per cent NaCl and 9 c.c. H_2O.....	50	38	19.4
2	1 c.c. 1 per cent KCl and 9 c.c. H_2O.....	50	38	23.0
3	1 c.c. 1 per cent $CaCl_2$ and 9 c.c. H_2O.....	50	38	23.0
4	3 c.c. phosphate mixture and 7 c.c. H_2O.....	49	30	23.0
5	100 mg. Fogelson's "Mucin" and 10 c.c. H_2O.....	52	32	16.0
6	20 mg. sodium salt of chondroitin sulphuric acid and 10 c.c. H_2O.....	50	38	13.0

N.B.—In all samples the reaction was adjusted with n/50 NaOH. Fogelson's "Mucin" in this experiment was extracted with absolute alcohol, which removed the soap and greater part of the phosphatides.

The above experiments therefore show that different preparations of gastric mucus not only combine with the free acidity of the gastric juice but exert a definite inhibitory influence on the peptic digestion. The analysis of the anti-peptic action of mucus, and certain observations made in this connection, inclined us to think that the inhibition of the action of pepsin by mucus is due to the presence in the mucoproteins of a prosthetic group, *i.e.*, mucoitin-sulphuric acid. Neither the protein part of the mucoprotein, nor the unsplit mucoprotein itself, seems to possess this property.

The first fact which we observed was that the sodium salt of chondroitin-sulphuric acid inhibited the peptic digestion much more strongly than Fraction 3 and Fogelson's "Mucin" (Table IV). In these experiments, instead of mucoitin-sulphuric acid, which was not obtainable in a sufficiently purified state, we used the sodium salt of chondroitin-sulphuric acid (kindly supplied by Dr. P. A. Levene of the Rockefeller Institute).

Table V gives a record of an experiment showing the effect of different substances on peptic digestion after 24 and 36 hours' incubation. In comparing the inhibitory action of sodium salt of chondroitin-sulphuric acid and Fogelson's "Mucin," it is interesting to note that not only the same amount of the former (sample 1) but even one-sixth of that amount (sample 2) produced a greater inhibition during the first 24 hours than Fogelson's "Mucin" (sample 3).

TABLE IV.

EXPERIMENT, APRIL 12, 1932.

Sample	*To 6 c.c. gastric juice, 6 c.c. H_2O and 3 c.c. n/50 NaOH added:*	*Acidity in c.c. n/10 NaOH before digestion*		*Acidity in c.c. n/10 NaOH after digestion*		*Peptic power, square of mm.*
		Total	*Free*	*Total*	*Free*	
Control		59	52	59	52	30
1	120 mg. sodium salt of chondroitin-sulphuric acid.....	61	42.5	61	42.5	9
2	120 mg. Fraction 3.....	61	45	62.5	41	15
3	120 mg. Fogelson's "Mucin"..	62	45	63	42	18.5

TABLE V.

EXPERIMENT, APRIL 14, 1932.

Sample	*To 6 c.c. gastric juice added:*	*Acidity in c.c. n/10 NaOH before digestion*		*Acidity in c.c. n/10 NaOH after 36 hrs. digestion*		*Peptic power, square of mm.*	
		Total	*Free*	*Total*	*Free*	*After 24 hrs.*	*After 36 hrs.*
Control	9 c.c. H_2O and 3 c.c. n/50 NaOH.....	58	52	58	52	16	42
1	120 mg. sodium salt chondroitin sulphuric acid and 12 c.c. H_2O.....	63	44	63	45	4	12
2	20 mg. sodium salt chondroitin sulphuric acid and 12 c.c. H_2O.....	61	51	61	51	9	25
3	120 mg. Fogelson's "Mucin" and 12 c.c. H_2O.....	64	46	65	44	12	27
4	120 mg. dissolved mucin, 6 c.c. H_2O and 6 c.c. n/50 NaOH.....	64	46	70	38	8.5	27
5	120 mg. submaxillary mucin and 12 c.c. H_2O.....	56	46	57	44	16	37

"Dissolved mucin" (Webster and Komarov[27]) inhibited the action of pepsin (sample 4) in the same degree as 20 mg. of sodium salt of chondroitin-sulphuric acid. After an additional twelve hours of incubation the end result of inhibition was practically the same for samples 2, 3 and 4. Very interesting results were obtained when mucin isolated from dog's submaxillary saliva (Komarov) was added to gastric juice. It produced no inhibitory effect during the first twenty-four hours of incubation, but quite a distinct one after an additional twelve hours. Similar results were found to prevail when highly purified surface epithelium mucus from alkaline gastric secretion (Komarov) was added to gastric juice. A feasible explanation of these facts may be that an unbroken mucoprotein compound does not possess antipeptic property, whereas the mucoitin-sulphuric acid which is gradually formed from mucin inhibits the peptic activity.

DISCUSSION

The data reported above show that different preparations of mucus from gastric mucosa possess an acid-combining property and also inhibit the action of pepsin. This latter property is exhibited independently of the capacity of the mucus to lower the acidity of the gastric juice to which it is added. It is highly probable that the substances responsible for this effect are of an organic nature, being represented by mucoproteins and chiefly by their derivatives, *i.e.*, mucoitin- and chondroitin-sulphuric acid.

From these experiments certain practical and theoretical conclusions may be drawn. From a practical point of view the use of properly purified mucus preparations in cases of gastric and intestinal ulcers may find justification in their neutralizing and antipeptic action. The use of mucoitin- or chondroitin-sulphuric acid in the form of a sodium salt might perhaps give more satisfactory results. However, it must be strongly emphasized that only thorough clinical investigation can finally determine the value of this new therapeutic measure.

From a theoretical point of view the antipeptic action of mucoitin- or chondroitin-sulphuric acid is of very great interest. Among other constituents the gastric glands secrete a mucoprotein soluble in acid gastric juice (Webster and Komarov[27]). Its secretion is under the control of the vagus nerve (Babkin,[4,5] Webster[26]). The greater the secretion of pepsin (the output of which is also regulated by the vagus), the greater is the amount of mucoprotein in the gastric juice. What is the relation of dissolved mucin to other constituents of the gastric juice, and what part does it play? Some authors (*e.g.*, Davis and Merker[10]) regard pepsin as a glucoprotein, though without bringing forward much supporting evidence. This is not in agreement with the recent findings of Northrop,[17] who obtained pepsin in crystalline form. It gave a negative Molisch test. Being extremely active (2.5 to 5 times more active than the commercial preparation of pepsin), it was unstable both in acid solutions and in the form of crystals (even in the ice-box). In connection with these facts it is interesting to note that in Pavlov's laboratory pepsin from gastric juice obtained by sham feeding of dogs was kept without any marked destruction of the enzyme for about a year.

Pekelharing[20] suggested that, in general, enzymes exist in the form of a complex compound, in which an active group is combined with protein. Would it not be more proper to suppose that in the gastric juice pepsin, although according to Northrop it is itself a protein, is secreted as a complex compound in combination with a glucoprotein or with mucoitin-sulphuric acid? Such a combination would result in diminished activity of the enzyme, but would also preserve it from destruction by hydrochloric acid and protect the mucous membrane from self-digestion.

SUMMARY

1. Gastric mucus possesses the properties of combining with acid and of inhibiting the action of pepsin in gastric juice.

2. The antipeptic action of mucus is chiefly due to the presence therein of some organic substance related to mucin, presumably mucoitin-sulphuric acid.

3. In commercial preparations of gastric mucus the presence of some inorganic constituents and soap, as well as other unidentified organic substances, contributes to the inhibitory action of the mucus on peptic digestion.

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CHOLECYSTITIS*

A BACTERIOLOGICAL AND EXPERIMENTAL STUDY

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THE following report is based upon the examination of 200 gall bladders removed at operation; 167 of these exemplified some grade of cholecystitis and 33 showed fibrosis of the gall-bladder wall without inflammatory cellular infiltration. Calculi were present in 65 per cent of the specimens of cholecystitis and in 31 per cent of the fibrosed gall bladders.

THE BACTERIOLOGY OF CHOLECYSTITIS

In all cases cultures were prepared from the gall-bladder wall inclusive of the mucous membrane. The selected portion of tissue, about 1 x 1 cm. in size, was washed in a stream of sterile saline solution, and finely minced before being transferred to a tube of glucose brain broth. In 138 cases additional cultures were prepared from the submucous layers of the gall bladder wall according to the technique described by Wilkie. In 106 cases the material in the gall bladder, bile or mucoid fluid, was also cultured. As soon as growth appeared in the primary cultures, subcultures were made on blood agar.

The following organisms were isolated.

1. A streptococcus, which grew in chains of medium length, which formed on blood agar small discrete, smooth, semi-translucent, non-hæmolytic colonies; which was killed by bile; and which on injection into the wall of a rabbit's gall bladder produced a progressive chronic cholecystitis. Thirty-eight per cent of the streptococci isolated were of this type.

2. A streptococcus which possessed all the above mentioned characteristics, except that the colonies on blood agar showed a green pigmentation. Five per cent of the streptococci isolated were of this variety.

Streptococci of both of these types are hereafter referred to as "typical" streptococci.

3. A streptococcus which grew in short chains or as a diplococcus, which formed on blood agar discrete, non-hæmolysing, colonies, rougher than those of the "typical" streptococci, being white and opaque in appearance; which was not killed by bile; and which failed to produce chronic cholecystitis when injected into the wall of a rabbit's gall bladder. Fifty-six per cent of the streptococci isolated were of this type. They are hereafter referred to as "atypical" streptococci.

4. *Streptococcus hæmolyticus.* This organism was isolated on one occasion only, and was regarded as a contaminant.

5. An organism which in the primary glucose broth cultures appeared as a small short-chained streptococcus or diplococcus, and when subcultured on blood agar grew as a diphtheroid bacillus, forming tiny greyish non-hæmolytic colonies. This organism was isolated in only a few instances; it was not further investigated.

6. *Bacillus coli.* Under this heading were classed all gram-negative motile bacilli which produced acid and gas in glucose and lactose media.

7. Staphylococci. Four per cent of the staphylococci isolated were of the *aureus* type, the remainder forming white colonies.

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Ninety-nine of the specimens were obtained from the Toronto General Hospital through the kindness of Dr. W. L. Robinson.